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# *Leveraging Algae: New Jobs for Our Oldest (and Smallest) Workforce*

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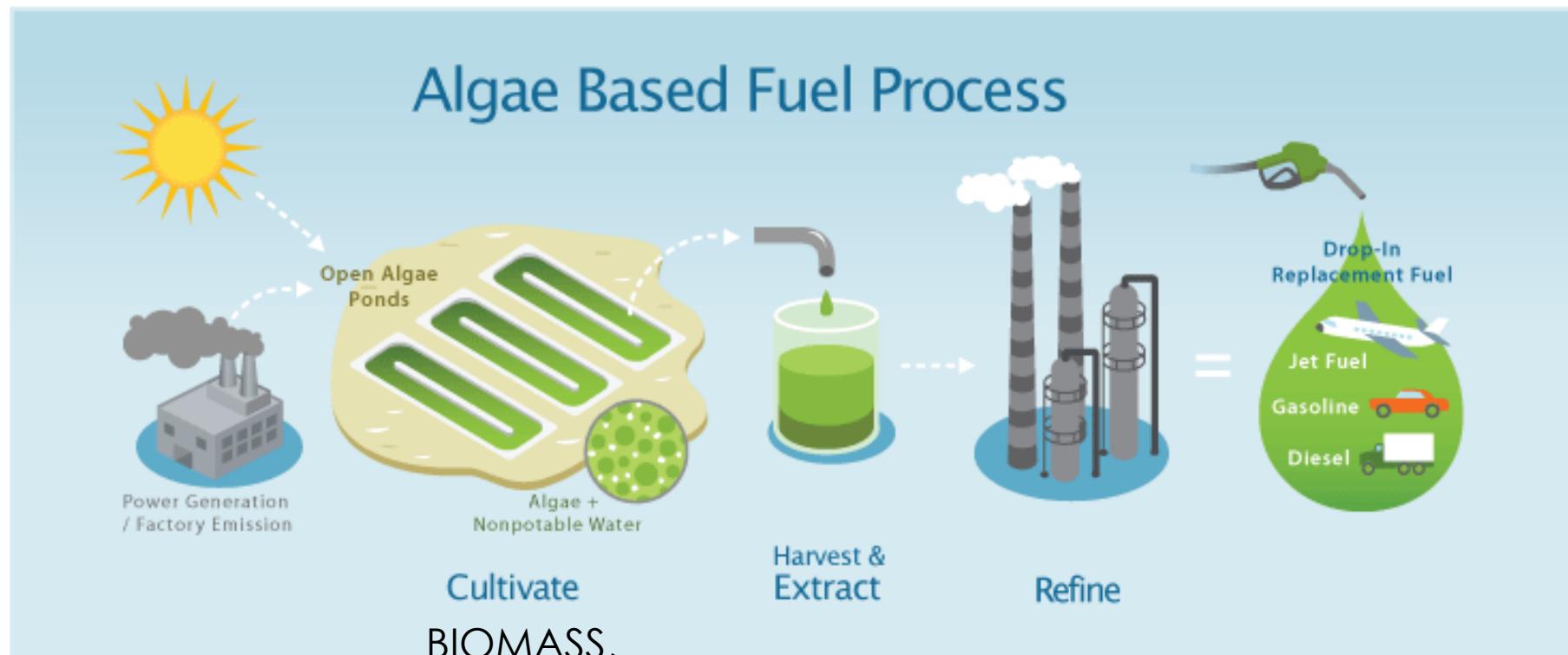
EST. 1943

# Outline

- ❑ Algal Biology and Evolution
- ❑ The Case for Algal Biofuels
- ❑ Modernization of the Workforce:
  - ❑ *Galdieria*- Relocation
  - ❑ *Chlorella* - Retraining
  - ❑ *Picochlorum*- Reduction

# Algal Biofuels

## How Does it Work?

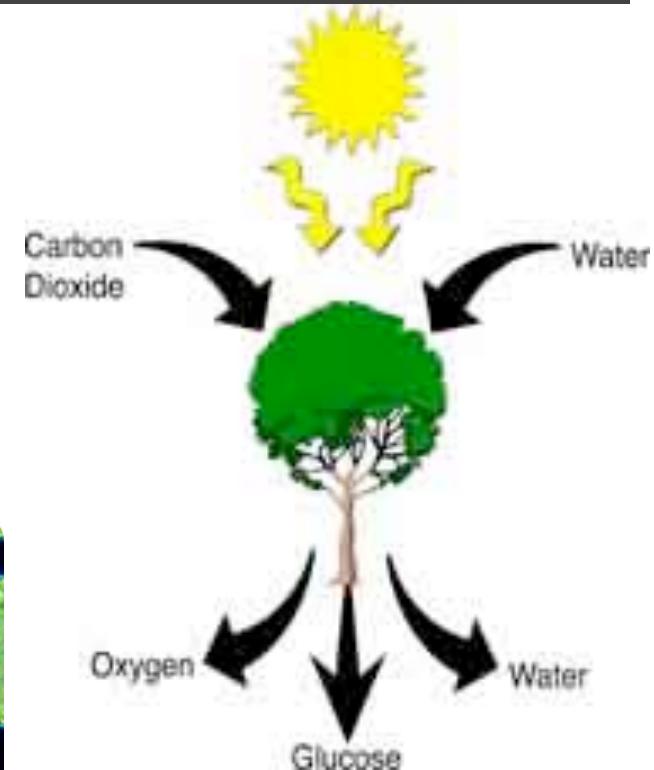
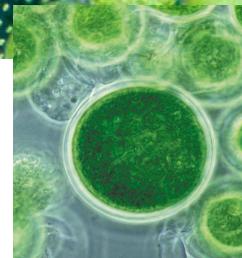
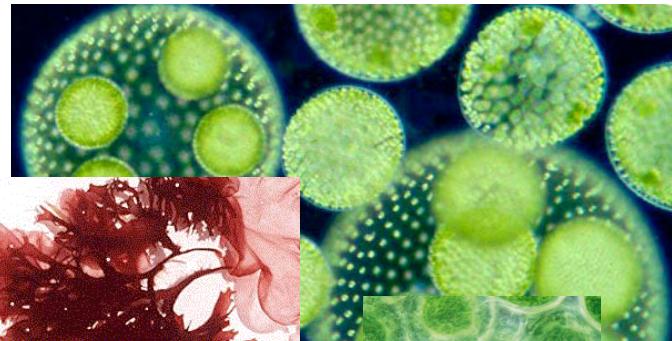


<http://www.sapphireenergy.com/green-crude/how-does-it-work/>

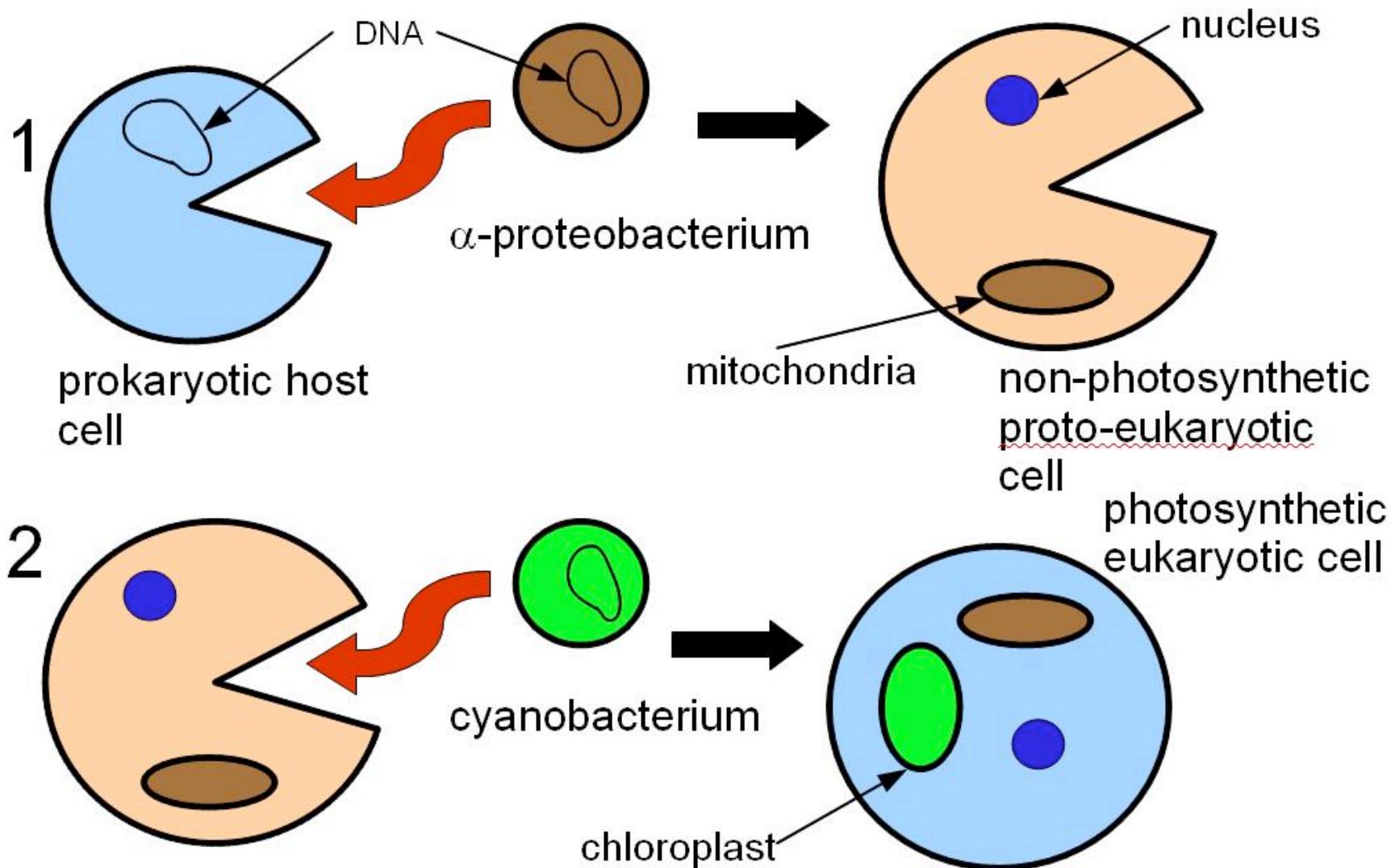
# What are Photosynthetic Algae?

- Eukaryotes
  - gain energy from light
  - Convert CO<sub>2</sub> into biomass

- Both multicellular and Unicellular



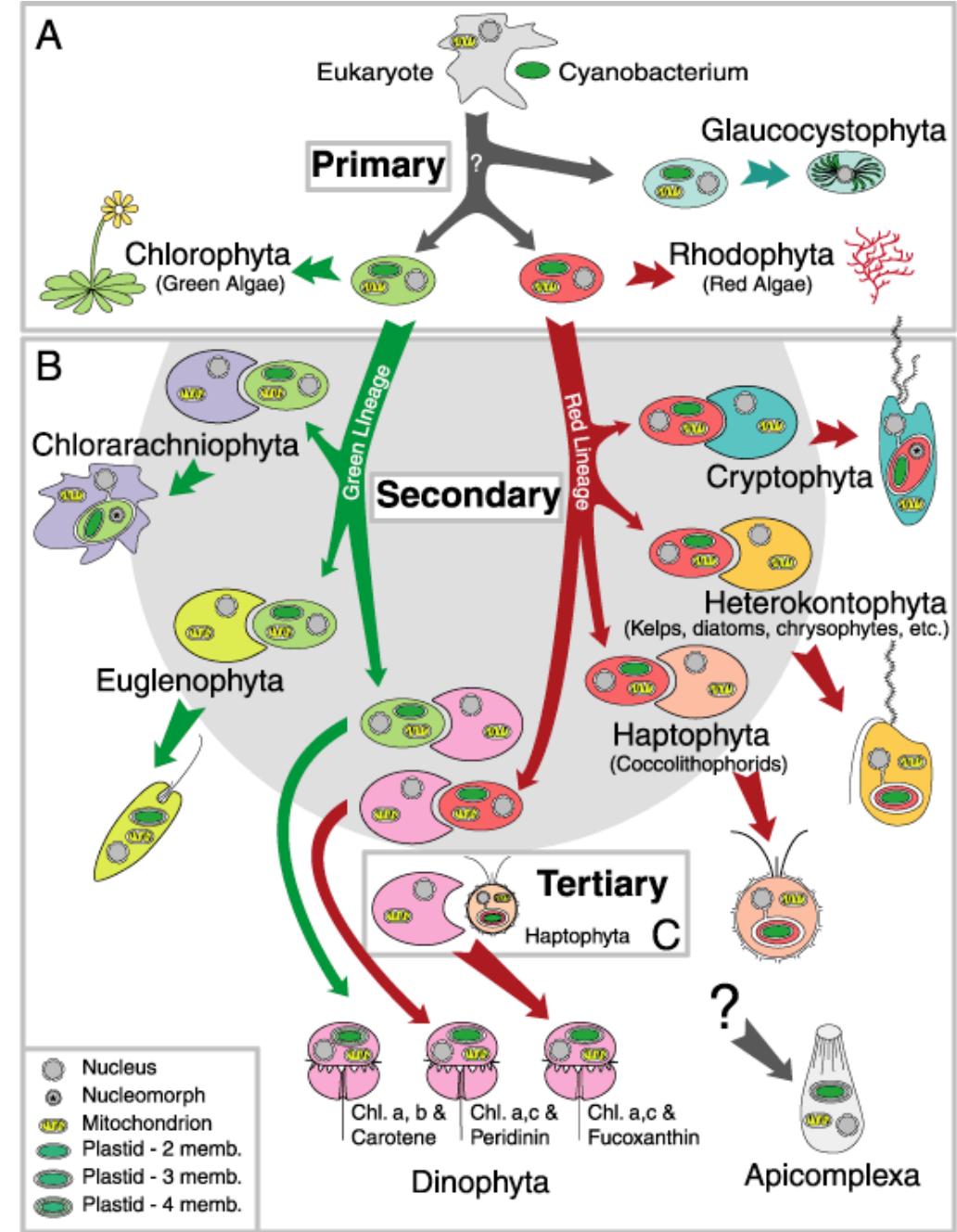
# Two endosymbiotic events c.2.7 bya



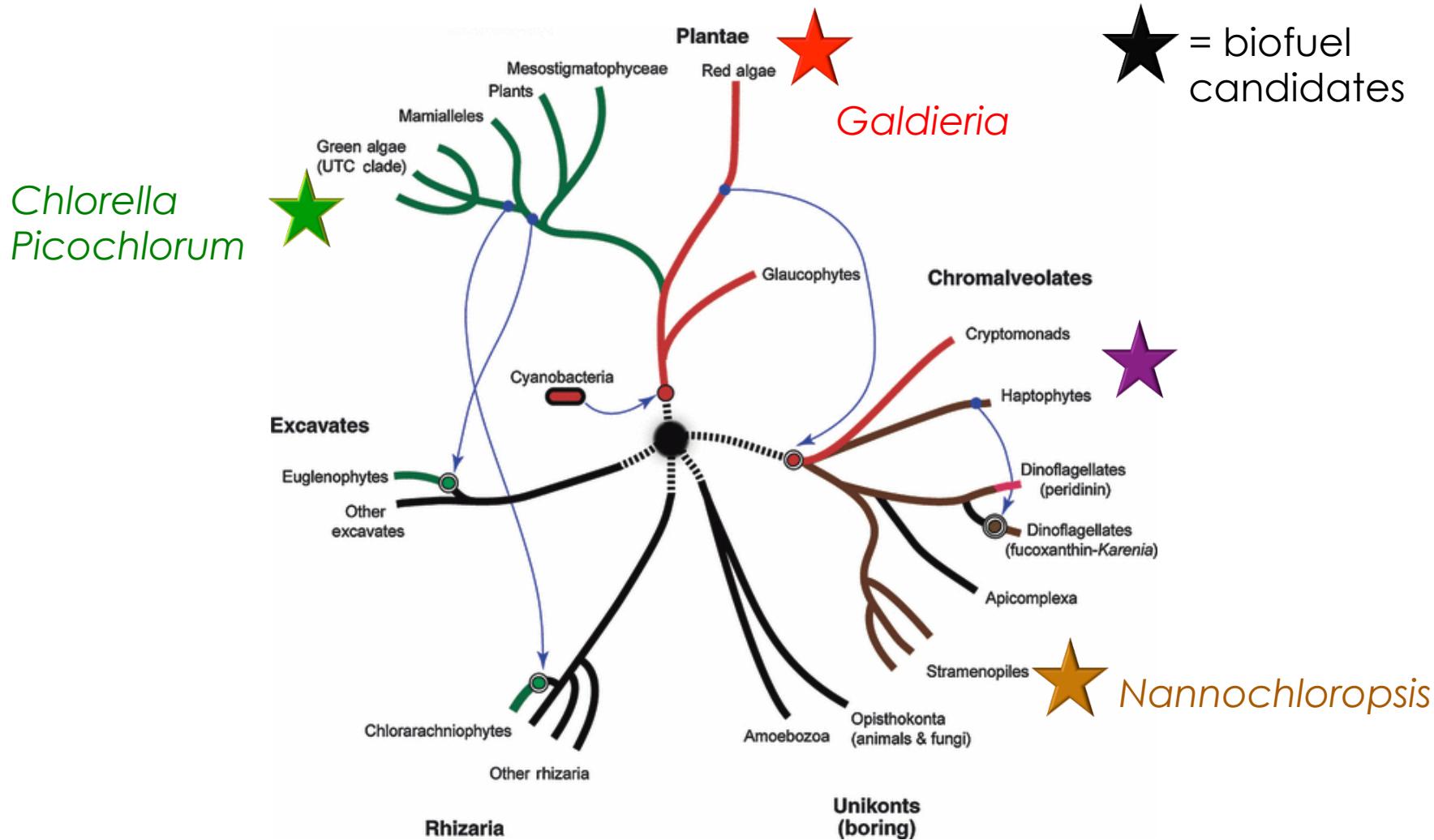
# Many Endosymbiotic Events Contribute to Algal Diversity

Common Names for Algae:  
“Red, Green, Brown, Yellow-Green, Golden, ...

“Blue-Green Algae” are  
**NOT** Algae: They are  
photosynthetic bacteria:  
(Cyanobacteria)



# Eukaryotic Tree of Life



# Prolific in Diverse Habitats



Arctic Sea Water



Alpine Freshwater Lakes



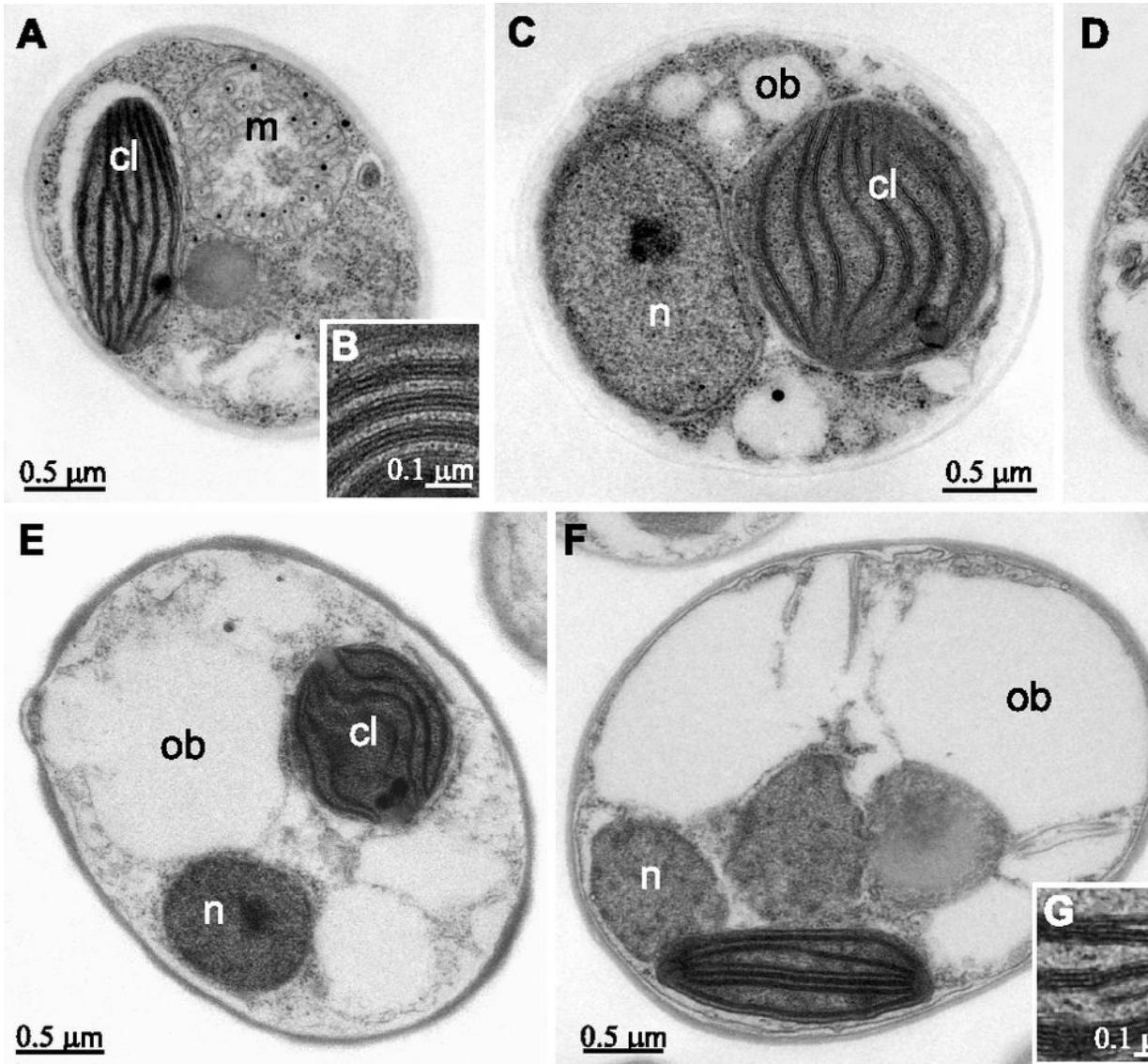
Hot Acidic Streams



Ditches, Ponds....

# Algae can store a lot of carbon

NITROGEN  
REPLETE



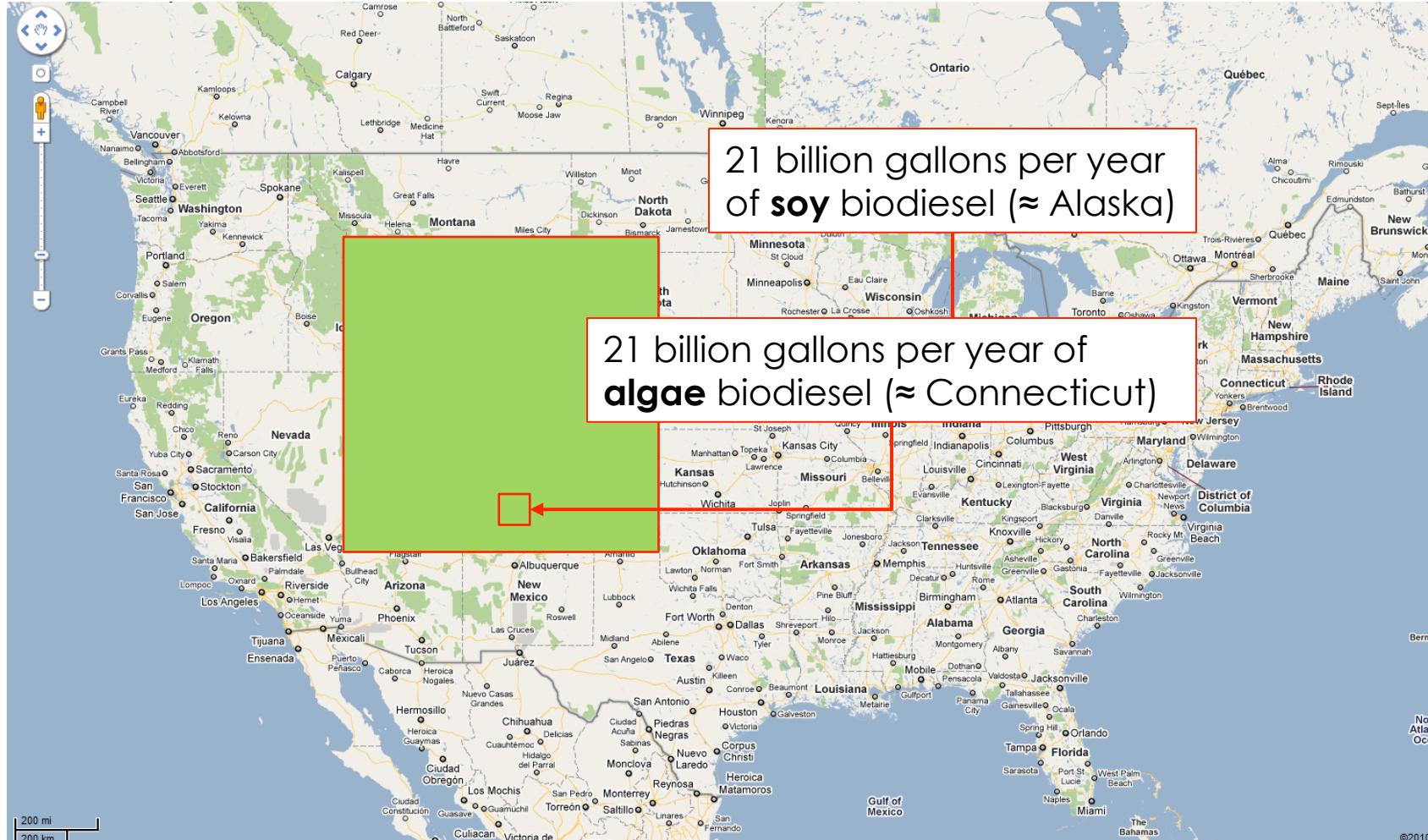
NITROGEN  
STARVED

# Other Advantages of Algae

- ❑ Grow Fast! (10 times more productive than land plants)
- ❑ Non-Competitive with Agriculture
  - ❑ Use of non-arable land
- ❑ Flexible Non-potable water sources
  - ❑ Wastewater
  - ❑ Saline and Brackish Water
- ❑ Products Plug into Existing Infrastructure
  - ❑ Biodiesel, Jet Fuel, Gasoline, Animal/Aquaculture Feed

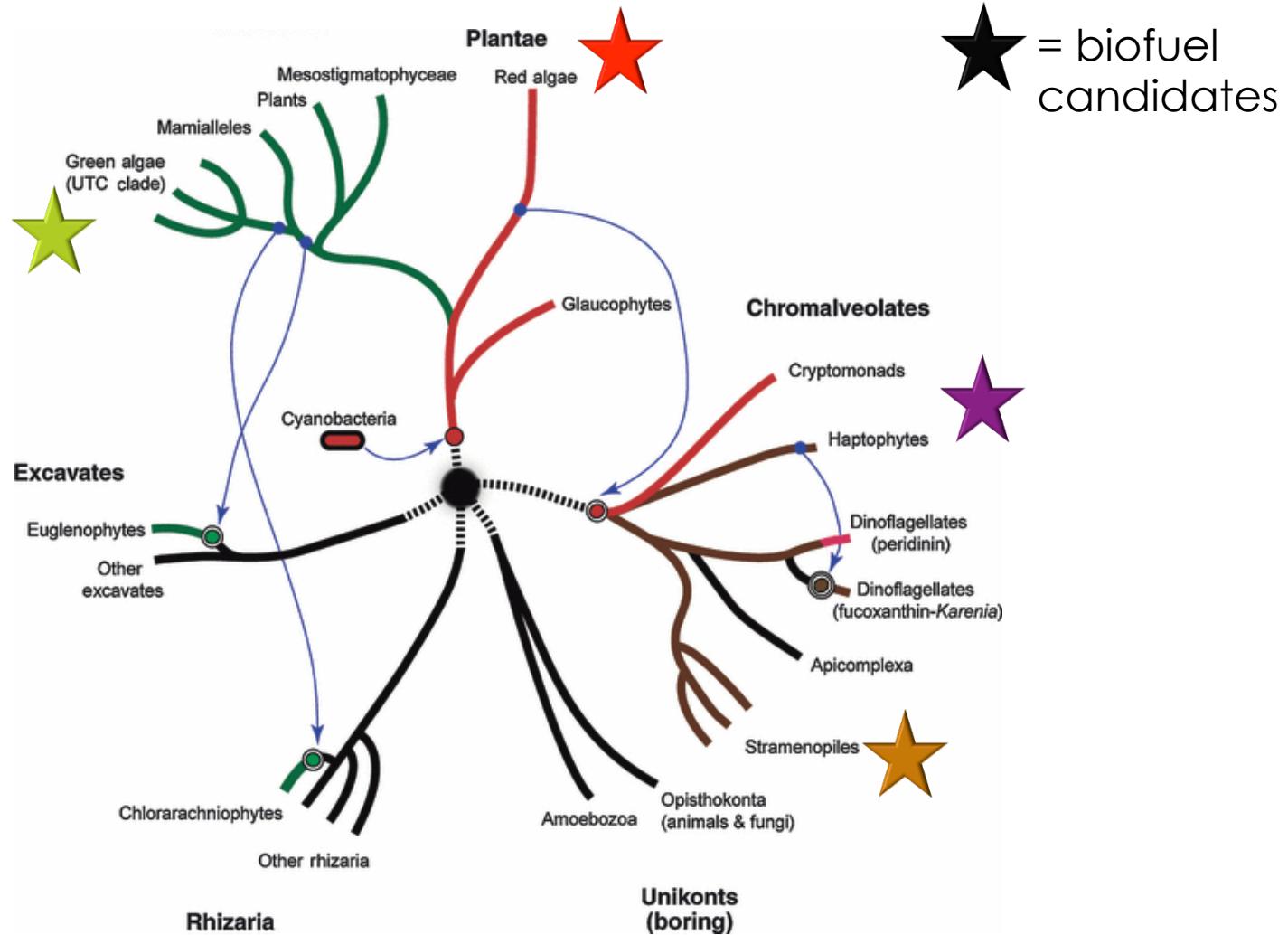
# Phototrophic Microalgae are Potentially Scalable

21 billion gallons per year of “advanced biofuels”  $\approx$  10% of U.S. liquid on-road fuel usage  $\approx$  how much cultivation area?

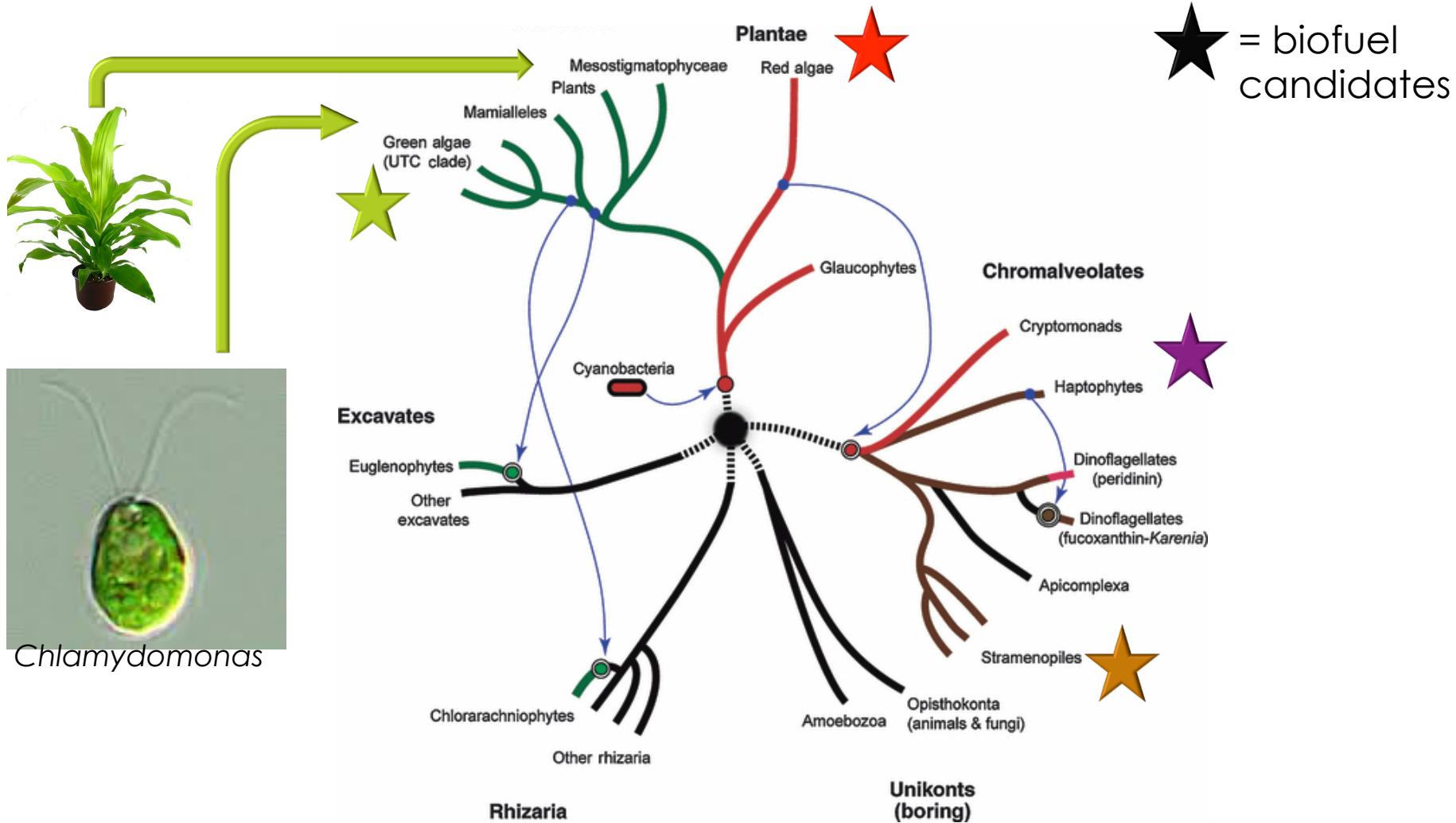


Personal comm., Anthony Marchese, Col. State University

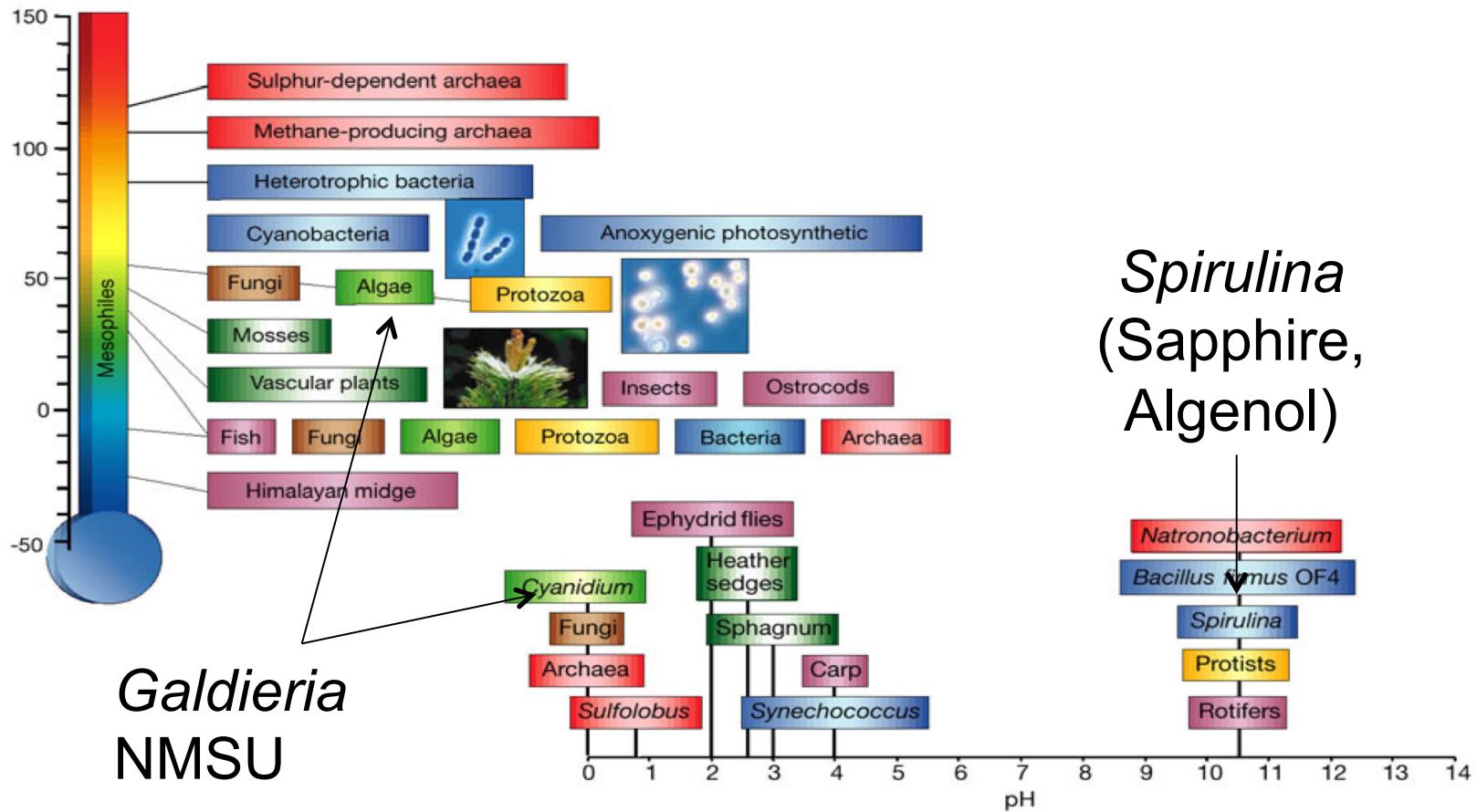
# Eukaryotic Tree of Life



# Eukaryotic Tree of Life



# Temperature and pH limits of life



Rothschild & Mancinelli *Nature* **409** , 1092 - 1101 (2001)

Courtesy of Pete Lammers, New Mexico State University

NAABB economic analyses predict that the major technological hurdles impacting economic success are; increasing biomass yield and reducing harvesting costs



	<b>Base</b>	<b>Scenario 1</b>	<b>Scenario 2</b>	<b>Scenario 3</b>	<b>Scenario 4</b>	<b>Scenario 5</b>
Products	Algae Crude Oil & LEA	Algae Crude Oil & LEA	Algae Crude Oil & Methane	Algae Crude Oil & Methane	Algae Crude Oil & Methane	Algae Crude Oil & Methane
Cultivation	Open Pond w/ Liners	Open Pond w/ Liners	Open Pond w/ Liners	Open Pond w/ Liners	ARID	ARID
Biology	Generic	Generic	Generic	GMO	Generic	GMO
Harvesting	Centrifuge	Electrocoagulation (EC)	Centrifuge	EC	EC	EC
Extraction	Wet Solvent	Wet Solvent	HTL-CHG	HTL-CHG	HTL-CHG	HTL-CHG
Nutrient Recycling	No	No	Yes	Yes	Yes	Yes
Biomass Production (Tons/Yr)	119,883	119,883	119,883	316,831	152,215	378,591
Crude Oil Production (Gallons/Yr)	4,679,762	5,095,741	15,006,224	43,184,240	20,747,054	51,602,173
Location	Pecos, TX	Pecos, TX	Pecos, TX	Pecos, TX	Tucson, AZ	Tucson, AZ

**James Richardson, TAMU, Myriah Johnson , TAMU, Meghan Downes, NMSU**

# *Galdieria sulphuraria*

- ❑ Isolated from Yellowstone National Park
- ❑ Metabolizes wide range of substrates.



Photo:YNP

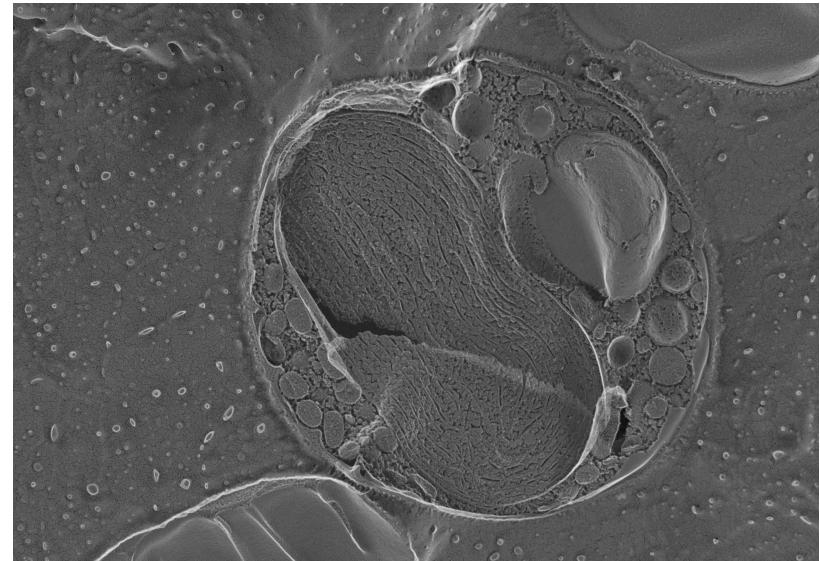
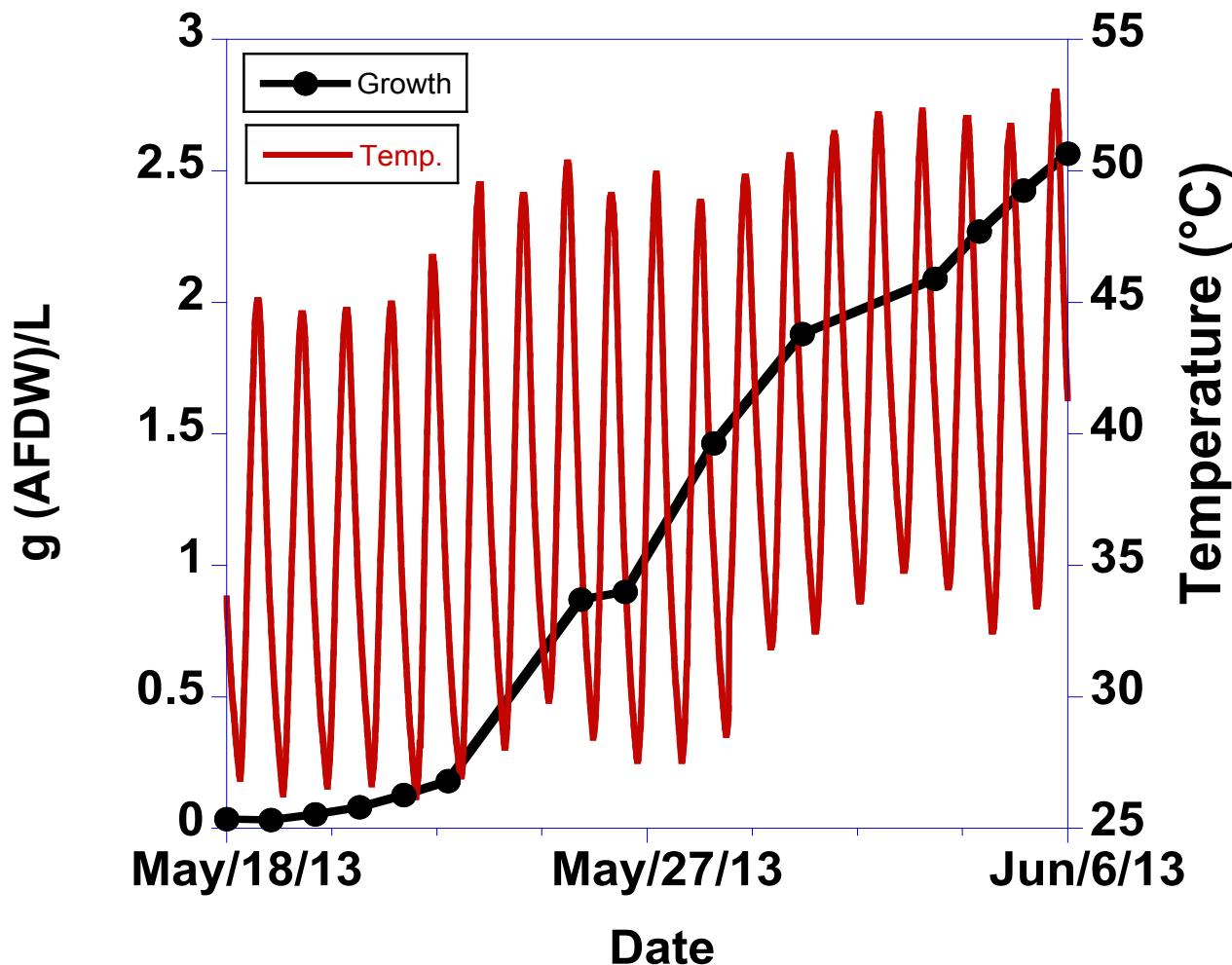


Photo:U. Goodenough

# Outdoor Growth of *Galdieria sulphuraria* in Enclosed Raceway

10 cm Depth, 5% CO<sub>2</sub> in Air, @ 2 L/min, O<sub>2</sub> in gas phase is ~30% during daytime

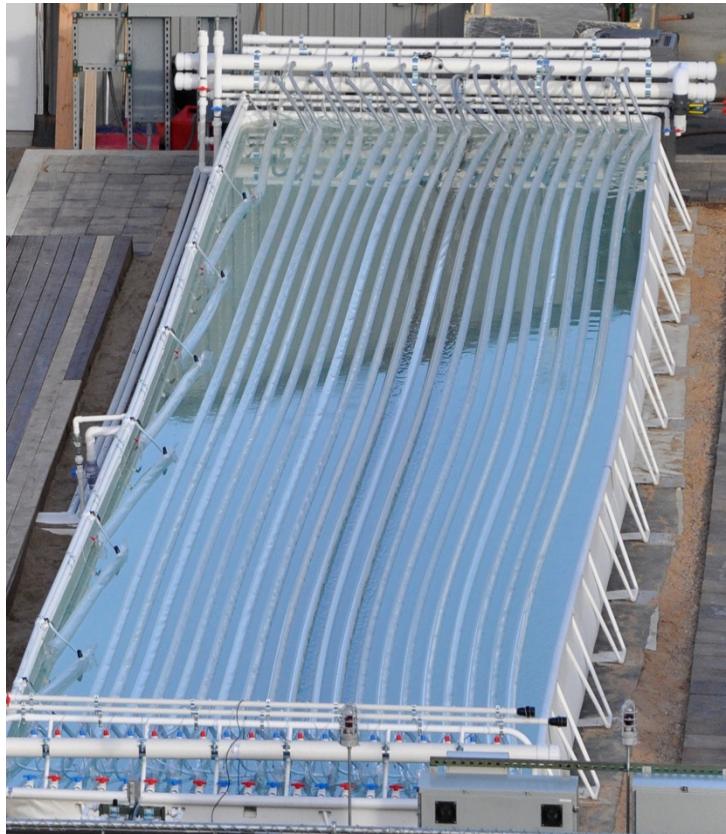


**0.165 grams/L/day**  
 $y = 0.165x + 0.159$   $R^2 = 0.978$

**16.5 grams/m<sup>2</sup>/day**

# Outdoor Production Systems

System 1: 20-200L panels in basin for temp control and year-around biomass production



Courtesy of Pete Lammers, NMSU

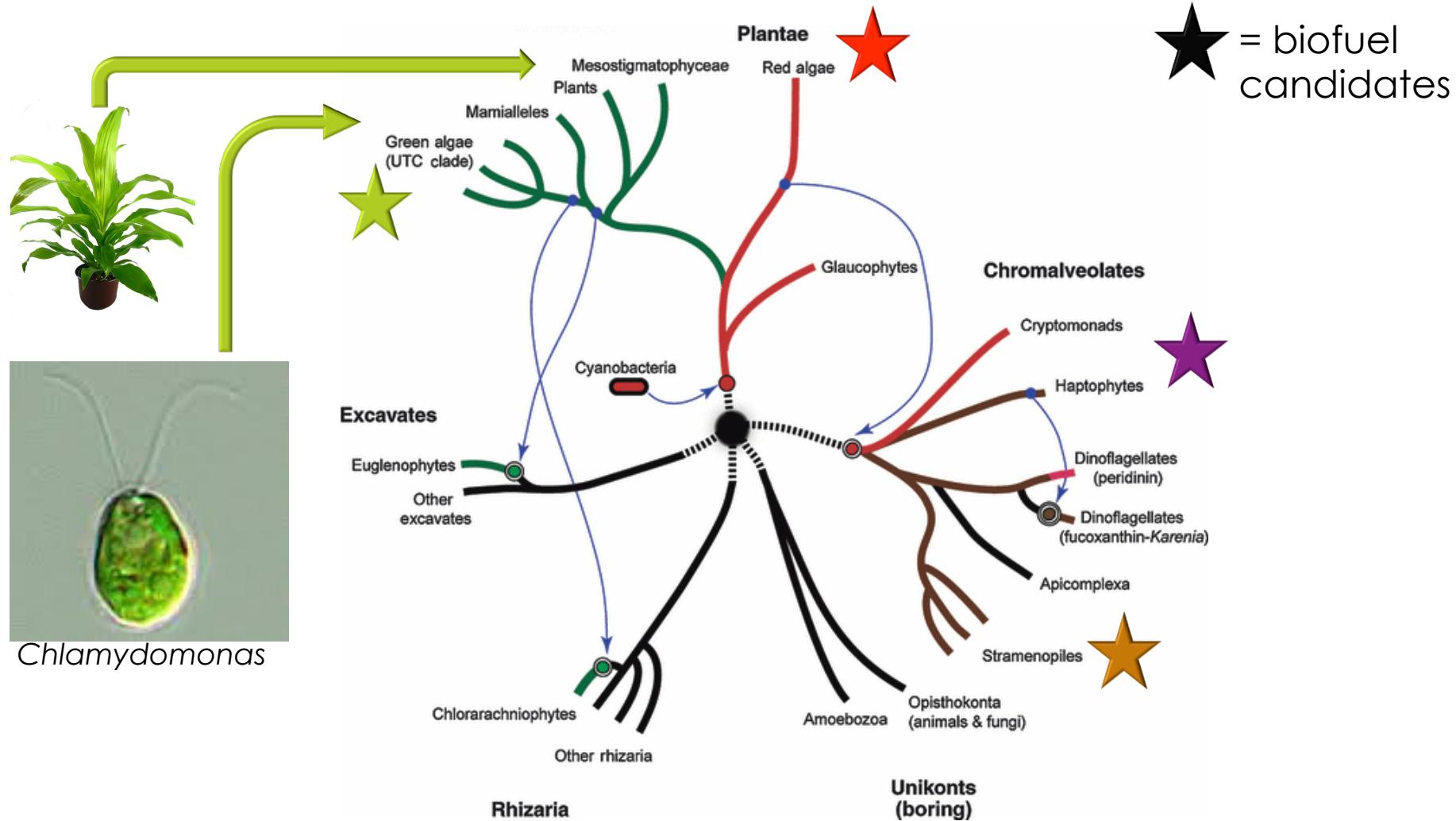
System 2: enclosed raceway design, passive solar heating, paddlewheel mixing



System 3: 4,500-L PBRs, waterfoil mixing, passive solar heating, condensation collection aids dewatering



# Eukaryotic Tree of Life



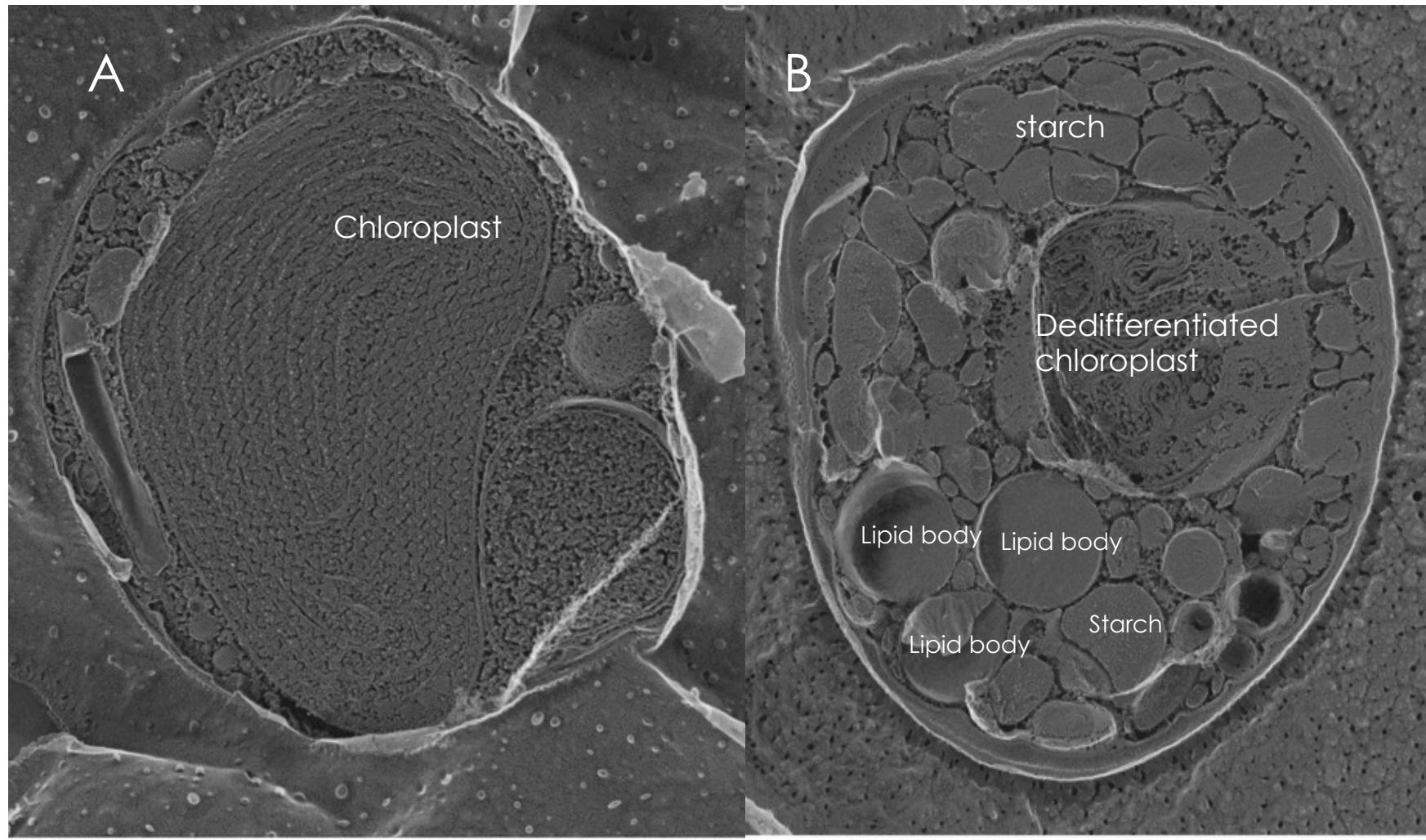
# *Galdieria sulphuraria*

- Unicellular red alga
- Acidophilic (pH 0-4) – self limiting – does not grow above pH 5
- Moderately thermophilic, maximum sustained temperature is 56°C
- Photoautotrophic and heterotrophic growth (up to 100 g/L)
- **10 successful batch cultures in ‘Sapphire-type’ PBRs (May-Oct, 2013). No crashes**

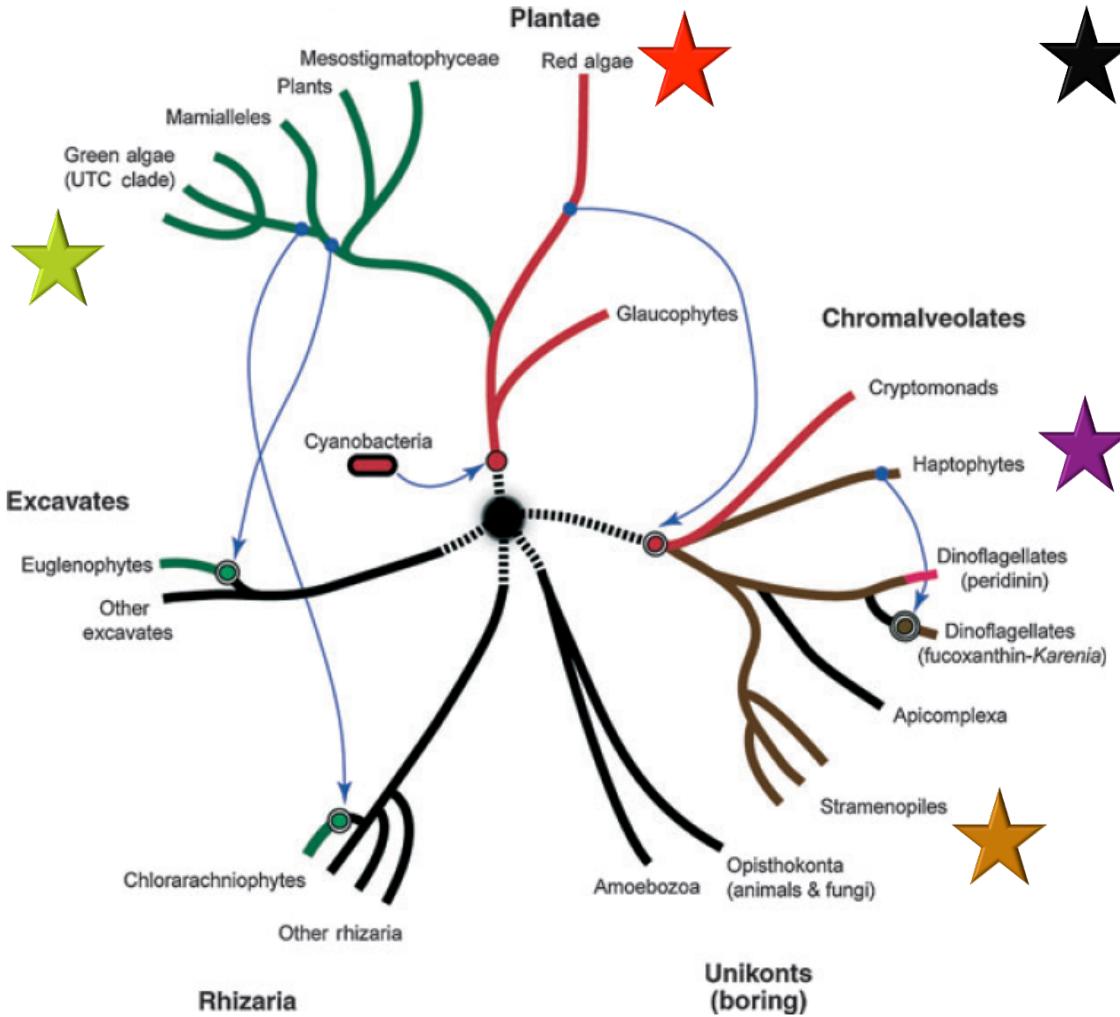
# *Galdieria sulphuraria*- Lipid and Starch Accumulation

photosynthetic growth stage

after nitrogen-limitation



# Eukaryotic Tree of Life



★ = biofuel candidates

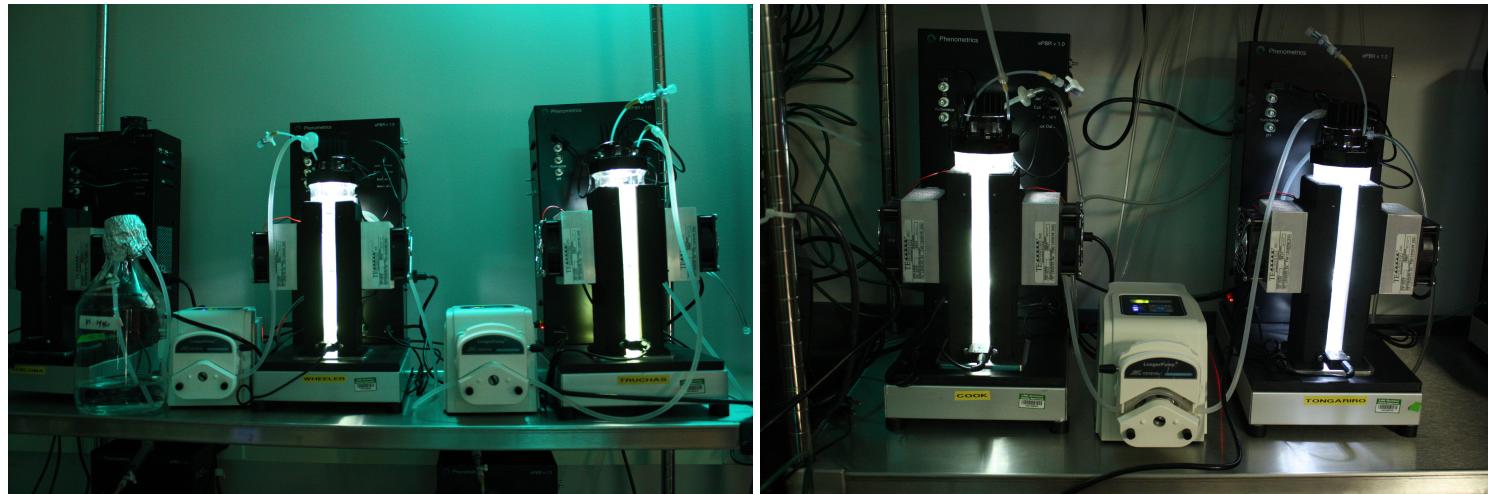
# Chemical Mutagenesis

- ❑ *Chlamydomonas* among lowest mutation rate recorded in eukaryotes (Ness et al. 2012 *Genetics* 192(4):1447)
  - ❑  $2 \times 10^{-10}$  mutation rate per cell division on agar plates
  - ❑ Mutation bias towards A/T
- ❑ Assuming a spontaneous mutation rate of 1/300 per replication and a doubling of  $10^7$  cells every 8 hours, we should have 100,000 possible mutations every day
- ❑ In order to obtain a more thermotolerant organism ( $50^\circ\text{C}$  or more), we will attempt chemical mutagenesis
- ❑ Ethyl methane sulfonate (EMS) increases spontaneous mutation rate up to 500 times
- ❑ Treat  $45^\circ\text{C}$  adapted cells with 100 mM EMS for 1 h (Ong et al. 2010 *Bioresource Tech* 101:2880)
  - ❑ Introduce into  $45^\circ\text{C} \rightarrow 50^\circ\text{C}$  and a  $50^\circ\text{C}$  photobioreactor environment

# Directed Thermoevolution

- ❑ *Chlorella sorokiniana*
- ❑ Increments of 2°C at a flow rate of 8 mL/hour (~200 mL/day)  
*1.5 months months*

39°C → 41°C → 43°C → 45°C → 47°C



# Acknowledgements

- ❑ **Picochlorum Adaptive Evolution and Nannochloropsis studies**
  - ❑ Scott Twary (LANL)
  - ❑ Taraka Dale (LANL)
  - ❑ Rose Ann Cattolico (U. of Washington)
- ❑ **Galdieria sulphuraria growth studies**
  - ❑ Pete Lammers (NMSU)
- ❑ **Thermoevolution of Chlorella**
  - ❑ Richard Sayre (LANL and New Mexico Consortium)
  - ❑ Amanda Barry (LANL)
- ❑ Current/Former Students
  - ❑ Jennifer Kwon (NIH)
  - ❑ Tumpa Arefin (UNM)
  - ❑ Juliette Ohan (LANL)
- ❑ Funding:
  - ❑ Dept. of Energy- Energy Efficiency and Renewable Energy Office
  - ❑ LANL- Laboratory Directed Research and Development

# Thank you!







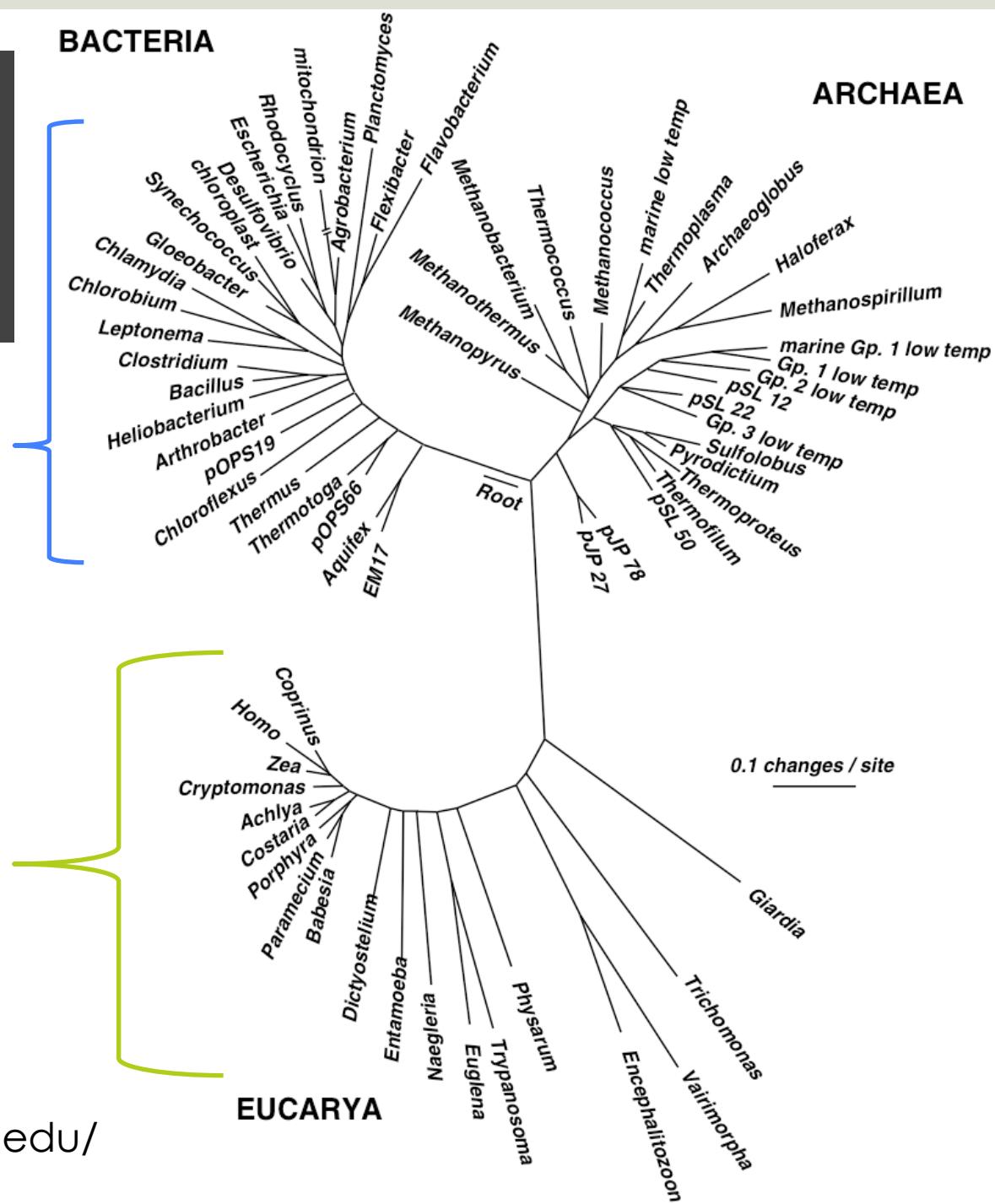
# The Tree of Life

**Cyanobacteria- Bacteria**

“Blue-Green Algae”

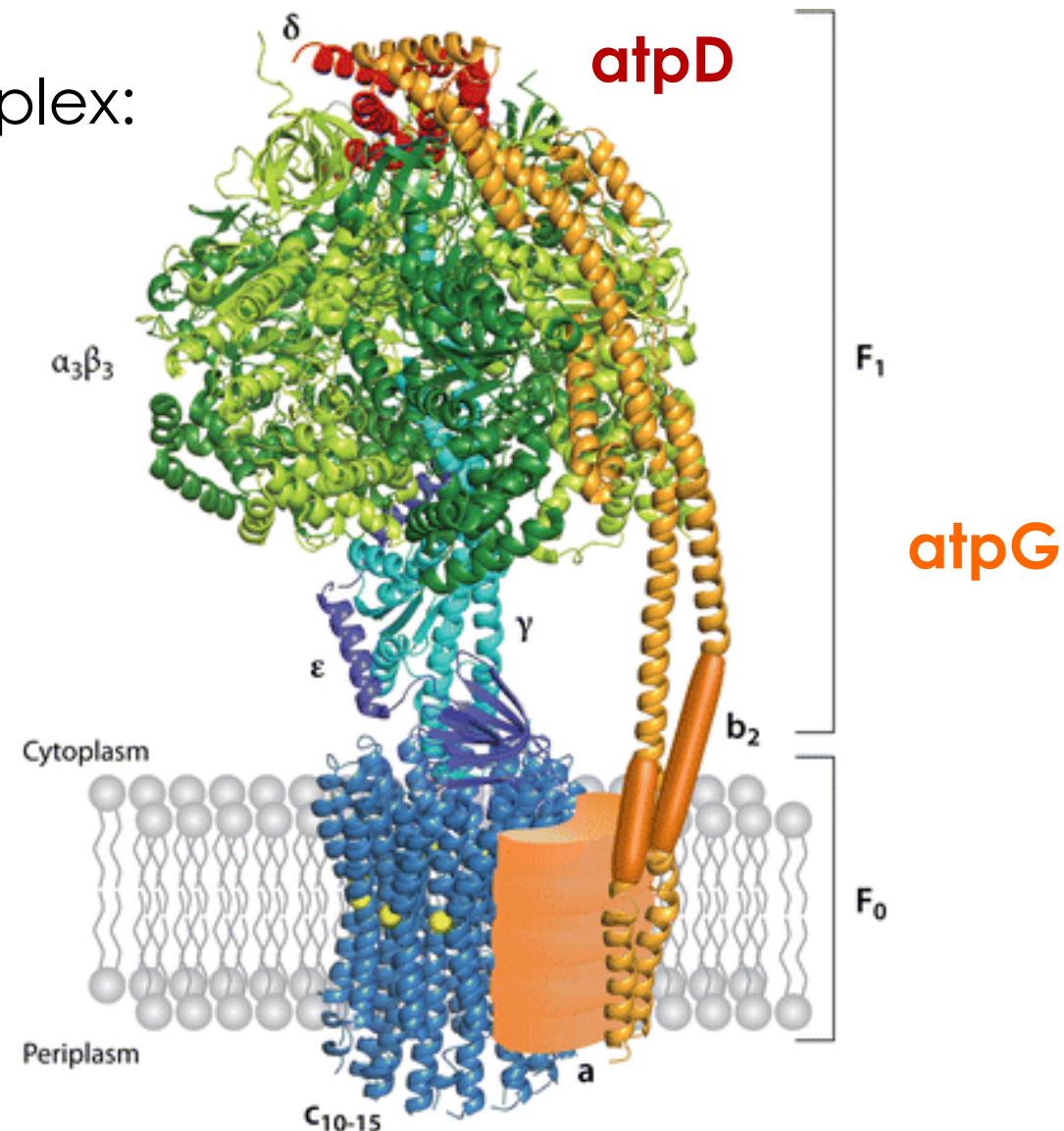
**Algae- Eukaryotes**

Common Names:  
“Red, Green, Brown,  
Yellow-Green, Golden,  
etc”



# ATP Synthase Complex:

Makes ATP!!



von Ballmoos C, et al. 2009.  
Annu. Rev. Biochem. 78:649–72

# ATP synthase subunit D

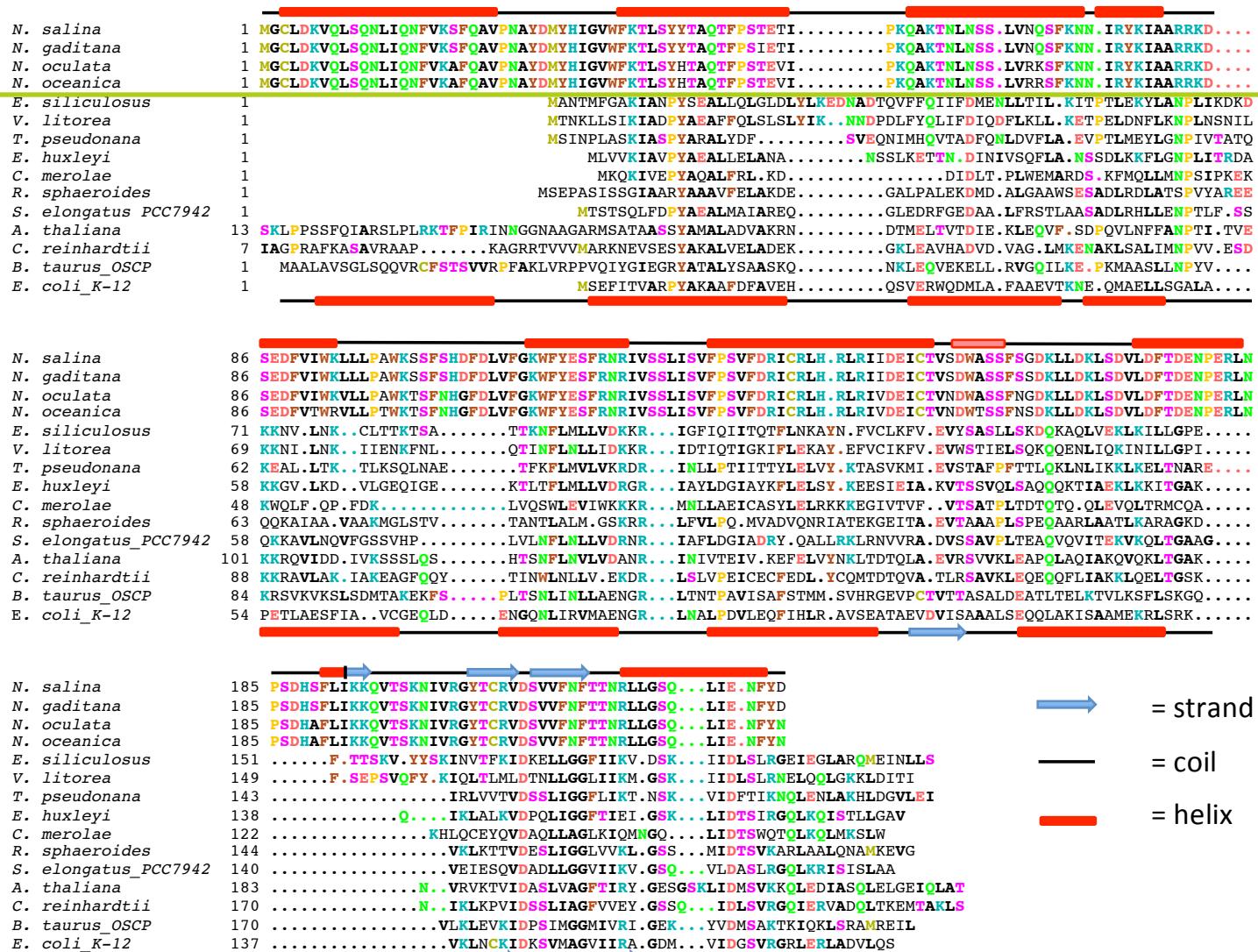
Nannochloropsis

Plants

Algae

Cyanobacteria

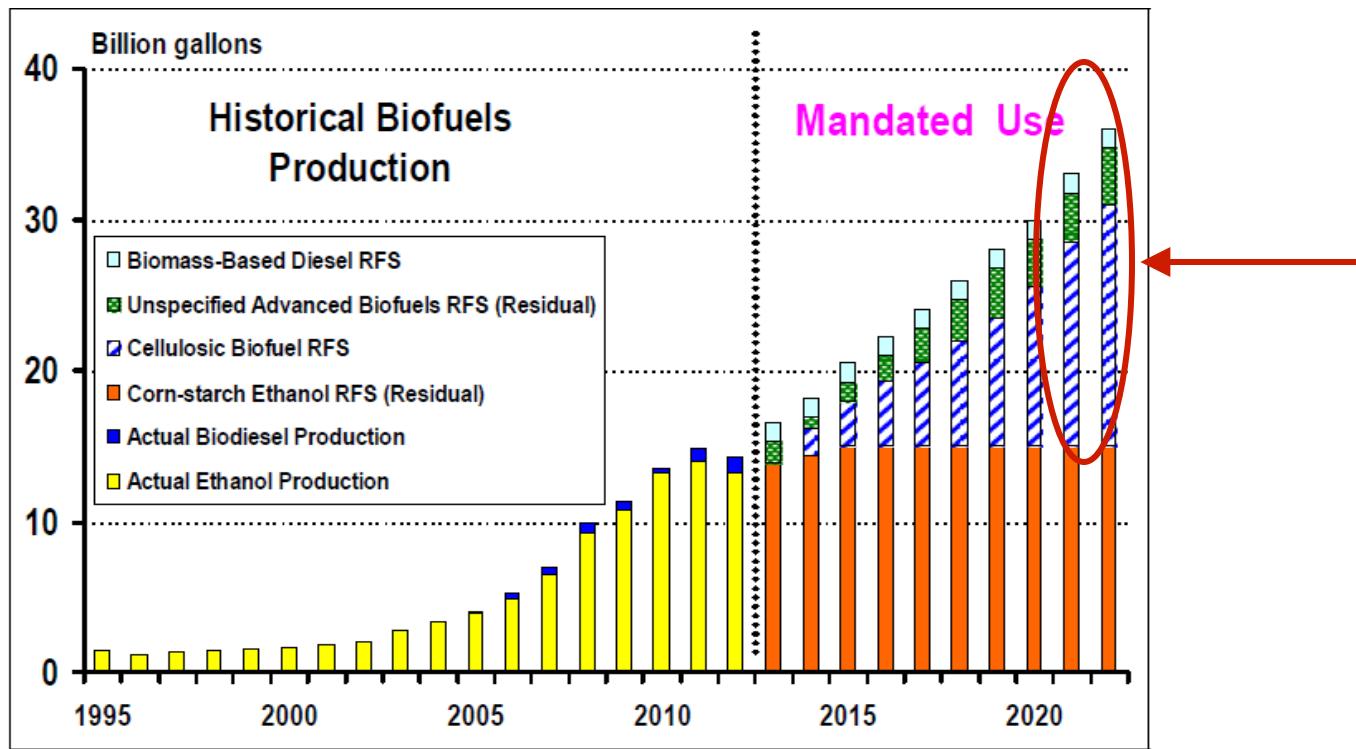
Bacteria



# U.S. Advanced Biofuels Mandate

## 21 billion gal/year by 2022

- The 2007 Energy Independence and Security Act (EISA) mandates the production of 36 billion gallons per year of biofuels by 2022
  - 21 billion gallons per year must qualify as advanced biofuels.



Source: Actual ethanol and biodiesel production data for 1995-2012 are from the Energy Information Agency (EIA), Department of Energy; the RFS2 mandates by category for 2013-2022 are from EISA (P.L. 110-140).

- Note: the U.S. consumes 300 Billion gallons per year of liquid fuels